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Circuit Behavior in the Development of Neuronal Networks

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S.B. Kater, Barbara C. Hayes

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The goal of this research has been to devise methods for accurately recording the activity of neuronal networks. The initial objective was to obtain multi-point recordings from neurons using extracellular electrical signals. For several reasons, this methods is now regarded with some skepticism: (1) Only neurons with very fast rising action potentials have been successfully recorded from because of the high-pass characteristics of extracellular electrodes (2) Only neuronal somata can be recorded from; (3) The primary interactions between neurons are on small neurites which are not routinely accessible by these methods; (4) Stimulation through these electrodes is quite possible, however, the efficacy of stimulation is only known when an alternative recording deveice is in place. Optical methods have been used with success for monitoring not just the extracellular events of neurons but also the intracellular changes in ionic activity. An extremely reliable method has been developed, using the dye Fura 2, for analyzing calcium currents, in circuit behavior. This work allows examination of the ensemble activity of specific sets of neurons given precise classes of input in order to assess the integrative qualities of the network and the specifi

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June 9, 1989

Dr. William O. Berry
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Bolling AFB, DC 20332-6448

Dear Dr. Berry:

Please accept my apologies for the form of report which you initially received. Not to make excuses, however, our research office indicated that a simple abstract was all that we needed. This seemed rather unusual and I am pleased to supply you with what I hope will be an appropriate final report. If there is anything I can do to increase the quality of this or change it in any way that you feel is necessary, please don't hesitate to tell me and on my return from Europe around the first of July, I will be happy to supply you with this promptly.

Again, my apologies.

Sincerely,

Stanley B. Kater, Professor
Director of the Program in Neuronal Growth and Development

SBK/ro

FINAL SCIENTIFIC REPORT
FOR GRANT AFOSR 87-0147

Project Title: Circuit Behavior in the Development of Neuronal Networks.

Principal Investigator: S. B. Kater, Ph.D.

Period of Support: 3/1/87 - 2/28/88.

(1) Concise Summary

This project centered around a single basic goal: examination of the activity of neural circuits from multiple locations in order to understand the rules governing the formation, maintenance, and remodeling of neuronal circuitry. Such an analysis would provide insights into the kinds of architecture employed by functional neuronal circuits; such architectures might be extrapolated to other computational needs. Potential approaches to this problem include (A) microelectrode recording which would give high resolution intracellular data (B) multiple extracellular electrodes which would give data on fast, transient events from multiple sites, and (C) optical methods which might give insights into many more sites but be somewhat restrictive as to the specific biological phenomena which they measured. We have examined all three of these during this year. Multiple microelectrodes, while giving the most precise data, are limited to measurement of the integration of all inputs to a neuron (as weighted by the electrotonic properties of individual neurons) and are limited by the number of microelectrodes that can be currently placed into the system. These data, which continue to provide extremely useful information, are severely limited when working with multiple elements. Large scale microelectrode arrays have been constructed in collaboration with a series of different laboratories. Although essentially identical qualitative results were obtained from all three sets of devices, each array or design system provided a variety of different advantages. All systems, we found, had some distinct limitations which arise from the nature of extracellular recordings. (1) Only neurons with very fast rising action potentials could be recorded from successfully because of the high-pass recording characteristics of extracellular electrodes. (2) Much to our regret, only neural somata could be routinely recorded from. (3) The primary interactions between neurons on small neurites as they frequently occur, were not routinely accessible by these methods. and (4) Stimulation of these electrodes is quite possible; the efficacy, however, of stimulation required an alternate recording device in order to confirm that such stimulation was supra-threshold. As an alternative of this, we have begun assessment of the third process for monitoring multiple electrical circuitry, namely, the use of intracellular optical markers which give an indication of the changes in biological function or activity. In particular, we have had, as described below, extremely good success using the calcium indicator, Fura 2 in looking at the interactions between neurons.

(2) Comprehensive List of Specific Research Objectives

(A) Monitoring circuit behavior through intracellular electrodes as a means of determining the kinds of interactions that occur between pairs, triplets, and larger ensembles of neurons from the snail, Helisoma, grown in cell culture. (B) The use of multielectrode extracellular arrays for recording from discreetly designed neuronal circuits

again grown in cell culture. (C) The use of fluorescent markers as a vehicle for monitoring changes in biological processes within individual neurons grown in cell culture in circuit arrays.

(3) Accomplishments

(A) We have been able to place individual identified neurons from the snail, Helisoma, in cell culture. Neurons known to participate in discreet interactions within the buccal ganglia of this organism can be removed and placed either as individuals in cell culture or as parts of specific circuit networks. For instance, an isolated neuron 4 or neuron 19 is grown in cell culture and its properties analyzed by intracellular microelectrode recording. Alternately, neurons 4 or 5 may be grown together or circuits can be composed of many neurons. We have now grown as many as seven homologous neurons, (i.e. seven neurons 5) together and as many as fifteen heterologous neurons together to form very complex circuit interactions in cell culture. We have monitored much of these interactions by intracellular microelectrode recordings. The data obtained can be highly accurate in that a microelectrode will integrate the total input to a neuron that is accessible to that point of recording according to the rules of electrotonic spread. Thus, if two neurons are interacting within a reasonable electrotonic distance, that interaction is observed by two microelectrode recording, one in the presynaptic and one in the postsynaptic neuron. The major conclusion from this approach has been that many more recording sites are required in order to really analyze the discreet processing capability of individual neurons and of large neuronal ensembles created in cell culture. To this end, one has to turn to a variety of alternate methods since it is not feasible to use, for example, twenty or thirty individual microelectrodes in an experiment such as the ones being undertaken.

(B) Extracellular microelectrode arrays offer promise in that they can be constructed using contemporary IC circuit design technologies. To this end, Ms. B. Hayes has made use of the Colorado State University Production Facilities in her initial design of a series of chips on which individual cells could be grown. It was found that the production facilities of other laboratories already in place could be combined with our experience in order to test the feasibility of the use of this kind of system for analyzing complex neuronal circuits. We have used devices made by Dr. G. Gross in Texas, we have collaborated with Dr. B. Wheeler at Illinois and constructed our own device, and we have made use of devices provided by Dr. Jerry Pine at Cal Tech. We have assessed the use of extracellular electrode arrays in recording from such circuits. The results, while suggestive and manageable, have distinct limitations in terms of the kinds of information that we would like to obtain. As stated in the summary, data from such arrays are limited to the same kind of limitations imposed by any extracellular recording device. The time constants of the system must be matched to the time constants of the signals and within the context of the biological signals we were measuring, recordings were limited to neurons with very fast rising action potentials. Furthermore, only large surface area elements could be used such that neuronal somata could provide signals: we could not obtain signals from the discreet, small processes where interactions between cells are clearly occuring. Thus, these chips provide only limited information in analyzing the behavior of experimentally constructed neuronal circuits.

(C) Optical dyes have recently grown in use in a variety of laboratories. We have made use of the calcium indicator, Fura 2 to measure what we think is one of the pivotal ions in integrative processes, namely intracellular calcium. We have found it possible with Helisoma neurons to employ a variety of loading methods of this compound and obtain reliable signals indicating the integrative capability of neurons over a vast array of the network. We have used a computerized optical scanning system in order to look at interactions between parts of neurons over large numbers of neurons. Individual neurons participating in discrete circuits can be analyzed in great detail. The resolution is not limited by the size of elements and the location of recording sight may be anywhere within the circuit proper. Furthermore, these dyes may be used on both large and small neurons. We have performed experiments using both Helisoma and rat hippocampal neurons in cell culture. Both give excellent signals and demonstrate the extremely good promise of this method as a vehicle for analyzing discreet interactions amongst even large ensembles of experimentally placed neurons within the context of functional neuronal circuitry.

Staffing

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